(type I, IIa, IIx, IIb) and polymorphic (I/IIa, IIa/IIx, IIx/IIb) fibers. Fiber MHC isoform content was determined by SDS-PAGE. The mean (± SE) pre- to post-eccentric change in force was −5.2 ± 0.5 kN/m^2 (n = 24) for type I fibers, −18.3 ± 1.5 (7) for I/IIa, −5.3 ± 0.7 (27) for IIa, −18.1 ± 2.3 (5) for IIa/IIx, −5.4 ± 0.6 (34) for IIx, −17.8 ± 1.1 (11) for IIx/IIb, and −5.7 ± 0.8 (24) for IIb. Multiple linear regression indicated that, independent of MHC content, greater pre-specific force was associated with a slightly greater post-force deficit (slope = 0.94). With pre-eccentric force held constant, no relationship was found between fiber V_o and post-eccentric force (p = 0.716) . In contrast, data were well described by a model using pre-eccentric force and MHC polymorphism as predictors (p < 0.001; r^2 = 0.97): post-force of monomorphic fibers = 0.94(pre-force) + 2.04 kN/m^2 post-force of polymorphic fibers = 0.94 (pre-force) - 12.15 kN/m^2 The significant difference (p < 0.001) in model y-intercepts reveals that, at similar pre-eccentric specific force, MHC polymorphism was associated with a 14 kN/m^2 greater eccentric-induced reduction in force vs. monomorphic fibers. We conclude that MHC polymorphism is associated with a heightened sensitivity to high mechanical strain at the level of the myofilament lattice or cytoskeleton.

686-Pos Board B486

Effects of R92 Mutations on Cardiac Contractile Function are Influenced by Changes in Myosin Heavy Chain Isoform

Steven J. Ford, Ranganath Mamidi, Jesus J. Jimenez, Jil C. Tardiff, Murali Chandra.

Mutations of cardiac troponin T (cTnT), many of which are found at its N-terminus (cT1), are associated with various forms of human heart disease. Within cT1, an amino acid at residue 92 (Arginine, R92) is a hotspot for point mutations. Previous studies using transgenic mouse models have shown that disease-related mutations R92 to Leucine (R92L) or Glutamine (R92Q) each influence contractile behavior of cardiac myofilaments. Because such cTnT mutations are often accompanied by an increase β-myosin heavy chain (MHC) expression in the failing human heart, we sought to determine whether the R92 mutation effects on cardiac contractile function are further influenced by a shift in MHC isoform content. Detergent-skinned papillary muscle fiber bundles were harvested from transgenic mice expressing R92L or R92Q against native α-MHC (R92L/α-MHC or R92Q/α-MHC) and transgenic mice expressing R92L or R92Q against predominantly β-MHC (R92L/β-MHC or R92Q/β-MHC). Constantly-activated fiber bundles from R92 transgenic and nontransgenic controls (α-MHC and β-MHC) were used to study how differences in MHC influence the effects of R92 mutations on Ca^{2+}- and length-dependent contractile activation. Our study shows that, concomitant with previous findings, R92 mutations against α-MHC result in a decrease in ATPase activity, increase in myofilament Ca^{2+}-sensitivity, and a decrease in cooperativity of myofilament force production. Furthermore, our study suggest that mutant R92 effects cardiac contractile dynamics in such a way that R92Q/α-MHC slows rates of crossbridge recruitment and crossbridge detachment and blunts the nonlinear effect that crossbridge distortion has on crossbridge recruitment. Interestingly, many of these R92 mutant effects were significantly influenced by expression of β-MHC. Collectively, these novel findings indicate a strong interaction effect and suggest that MHC structure further influences how cTnT regulates functional and dynamical aspects of cardiac contractile activation.

687-Pos Board B487

Static Stiffness in Slow and Fast Mouse Muscle Fibers Expressing Different Titin Isoforms

M. Angela Bagni, Barbara Colombini, Marta Nocella, Giulia Benelli, Joseph Bruton, Giovanni Cecchi.

We showed previously (Bagni et al., 2002) that most of the increase of muscle fiber stiffness during the early phases of a tetanic contraction is due to a non-crossbridge sarcomere component whose stiffness (called static stiffness) increases after stimulation with a time course very similar to the internal Ca2+ concentration. This led us to speculate that Ca2+ concentration, in addition to promote crossbridge formation, could also leads to a stiffening of a sarcomere structure, identified with the titin filament, either directly or through a titin-actin interaction leading to the observed sarcomere stiffness increase. According to this hypothesis, it is expected that static stiffness has different properties in muscles expressing titin with different mechanical properties. Therefore we compared the static stiffness values in soleus and EDL adult mouse muscles, which express titin isoforms with long and short PEVK segment, respectively. Considering that Ca2+ binding to E-rich motifs in the PEVK segment increases its bending rigidity, the higher proportion of these motifs in EDL compared to soleus is expected to lead to a greater static stiffness in EDL. Our results showed that in agreement with the titin hypothesis, the static stiffness measured in single fibers at 25°C was more than five times greater in EDL than in soleus and about two times greater than previously reported on FDB muscle. The static stiffness time course in EDL was about the same as in FDB but slightly faster than in soleus, and it became much faster at 35°C in both EDL and soleus similarly to tension time course. These results are in agreement with the idea that static stiffness depends on the increment of titin stiffness due to the interaction between Ca2+ and E-rich motifs in PEVK segment.

688-Pos Board B488

A Novel Approach to Study Mechanical Ventilator-Induced Diaphragm Muscle Weakness

Guillaume Renaud.

Mechanical ventilation (MV) is widely used clinically, despite being lifesaving, several evidences point out that prolonged MV induces diaphragm muscle weakness. Until now, most studies have focused on the impact of early MV (up to 18h) in rats . Long-term changes remain unknown. In our lab, we have developed a unique model allowing to keep a rat mechanically ventilated for up to 3 weeks. Therefore, in the present study, we looked at the effect of 2 weeks of MV and immobilization in rats. The rats were divided into 4 groups according to MV and immobilization duration : control, 1-4 days, 5-8 days and 9-14 days. Cross-sectional area (CSA) and specific tension (force normalized to CSA) were measured in maximally activated (pCa 4.5) skinned single fibres set at a sarcomere length of 2.6 µm. The specific tension was significantly reduced in the second, third and fourth group compared to control ($p<0.001$). On the other hand, the CSA remained unchanged in the second group compared to control and was significantly reduced in groups 3 and 4 ($p<0.02$ and $p<0.001$ respectively). This tends to indicate that early loss in specific force is likely to be due to post-translational modifications of the contractile proteins rather than up-regulated proteolysis. Further experiments to analyze myosin speed and force using a modified single fibre *in vitro* motility assay as well as myosin content using a PLA™ based assay will be presented at the meeting.

689-Pos Board B489

Mouse Intact and Skinned Cardiac Myocyte Mechanics: Crossbridge and Titin-Based Stress in Unactivated Cells

Methajit Methawasin, Nicholas M.P. King, Joshua Nedrud, Charles S. Chung, Michiel Helmes, Henk L. Granzier.

We investigated the contribution of actomyosin interactions in unactivated intact and skinned cardiomyocytes in physiologic conditions.

A carbon fiber based cell-attachment system was used to measure the diastolic stress-sarcomere length (SL) relation of murine *intact* cardiomyocytes, before and after the addition of actomyosin inhibitors (BDM or blebbistatin). Stress was measured during the diastolic interval of twitching myocytes that were stretched at 100% length/s. Diastolic stress increased nearly linearly from 0 at SL 1.85 µm to 4.2 mN/mm^2 at SL 2.1 µm. Actomyosin inhibitors lowered diastolic stress by ~1.5 mN/mm^2 at SL 2.1 µm (~30% of total), suggesting that during diastole actomyosin interaction is not fully switched off. Stretch-hold-release studies on *skinned* cardiomyocytes showed that as temperature changed from 24°C to 37°C, there was shortening of slack SL (from 1.90 ± 0.01 µm to 1.89 ± 0.01 µm) and increasing of both peak stress (~35%) and steady state stress (~26%). Shortening of slack SL and increasing stress could be inhibited by blebbistatin. This suggests that at physiologic temperature, crossbridge cycling takes place which contributes to diastolic stress. To extend this further, calcium sensitivity of *skinned* cardiomyocytes was studied under conditions that simulate physiologic diastole: 37°C, presence of Dextran T500 to compress the myofilament lattice to the physiological level, and [Ca^{2+}] from below to above 100 nM. Mean active stress was increased at [Ca^{2+}] >55 nM (pCa 7.25) and was ~0.7 mN/mm^2 at 100 nM [Ca^{2+}] (pCa 7.0) and ~1.3 mN/mm^2 at 175 nM [Ca^{2+}] (pCa 6.75). The presence of active stress at pCa 7, which is a physiologic Ca^{2+} concentration of cytoplasm during diastole, confirms the contribution of crossbridge cycling to diastolic stress. These findings are relevant for understanding diastolic function and for future studies of diastolic heart failure.

690-Pos Board B490

Characterization of Single- and Double-Headed Non-Muscle Myosin IIB Heavy Meromyosins using Single Molecule Techniques

Attila Nagy, Yasuharu Takagi, Earl E. Homsher, Davin K.T. Hong, James R. Sellers.

Non-muscle myosin IIB (NMIIB) is a cytoplasmic myosin, which plays important role in cell motility by maintaining cortical tension. It forms bipolar filaments with ~14 myosin molecules on each side of the bare zone. Our previous studies showed that the NMIIB is a moderately high duty ratio (~20-25%) motor. The ADP release step (~0.35 s-1), of NMIIB is only ~3 times faster than the rate-limiting phosphate release (0.13 s-1), and as a result acto-NMIIB has the highest ADP-affinity reported so far for the myosin superfamily